FOOD SHORATGE IN COLONIES AFFECT THE DEVELOPMENT OF FORAGER HONEY BEES (A*PIS MELLIFERA* L.) APIDAE; HYMENOPTERA

Hafiz Khurram Shurjeel*[1], Yusra Yasmin[2], Sadia Khanum[2], Sundas Asghar[2], Nadia Saeed[3], Ambreen Akram[4], Shazia Iqbal[5], Nasir Khan[6], Khayam[7], Riaz Hussain[1], Murad Ali[7]

[1]The University of Agriculture Swat-Pakistan
[2]University of Lahore- Pakistan
[3]Government Post-graduate College, Mandian, Abbotabad- Pakistan
[4]Federal Urdu University, Karachi- Pakistan
[5]University of Sargodha- Pakistan
[6]Agriculture Research Institute, Mingora-Pakistan
[7]Islamia College Peshawar-Pakistan

Correspondence*: shurjeel_38@yahoo.com

ABSTRACT

Honey bees (A*pis mellifera* L.) are imperative and pollinators and require sufficient food for their survival. The worker with great pollen and nectar collecting capacity are considered the proficient and comparative to others. Food requirement in bee colonies triggers few colony strength characteristics. Apart from various perspectives such as genotype and approximate generous assets, predominantly forager's triggers the number of hatching through providing sufficient food. The current study was conducted to check the effect of food shortage in the honey bee colonies. In the first experiment four colonies were used; one colony was named as a fed colony (control treatment) and other three colonies were declared as starved colonies. In the second experiment, two colonies; one as fed and other as starved were used. In the third experiment, three colonies; one as fed (control treatment), one as starved and one FBSC colonies were used. The mortality was more in the starved colonies than in fed ones in all the experiments with the significant values showed by Chi- square model $df= 3, X^2= 16.04, p= 0.0001, df= 3, X^2= 1448, p= 0.0004$ and $df= 3, X^2=$ 28.82, $p < 0.00001$ respectively. Therefore it is concluded from the findings that food shortage in the colony increase mortality and bee behavior.

INTRODUCTION

Honey bees (A*pis mellifera* L.) are imperative social insects and pollinators. Since, bees are of great important for environment, economy and health, these are considered as an ideal model for many biological and physiological studies. It is believed that every factor play a role in bee' health, although no evidence has been found to be the only factor of population decline worldwide. Bees as a pollinator and forager get across to transform the advantageous interface and an influential trade complex (Traveset and Richardson 2006). They may uncomplicatedly get better home-produced plant wellbeing through pollination (Kato et al. 1999) and vary from the tamed honey bees in structural prototype of transfer and pollen quantity (Freitas 1997; Freitas and Paxton 1998). worker bees with great pollen and nectar collecting capacity are considered more proficient comparative to others (Cane and Schiffhauer 2001). Food requirement in bee colonies triggers few colony strength characteristics. Apart from various perspectives such as genotype and approximate generous assets, predominantly forager's triggers the number of hatching through providing sufficient food.

Adult worker bees vigor the colony through food provision and cannot survive for a prolong time exclusive of feeding because they get deficiency of carbohydrate. Indispensable nutrients; macro-nutrients and sole nutrients are compulsory for bees' survival (Jung and Berget, 2011). Similarly, numbers of forager bees in a prefecture are compulsory for an ecosystem. Foraging behavior of bees is association flanked by colony and common environment and consequently, various in-colony and out-colony features influence this behavior. Searching behavior of bees isn't productive regarding circumstances and plants pollination nevertheless some additional constructive circumstances are included for the execution of smooth productive colony health. All foragers do not have similar manifestation of collecting flora during their field visits. There is an enormous divergence happening amongst foraging prototype of bees. It has been observed that bumble bees are more hectic as compared to honey bees since they bring together the pollen and nectar from field as a minimum two times from numerous plant species than the honey bees commonly accomplish (Leonhardt and Blüthgen 2012). Honey bees are more hybrids in visiting plants during one foraging and demonstrate more constant foraging behavior while bumble bees during their one foraging round collect pollen from only six different plant verities (Kratochwil and Kohl, 1988).

Quantity of honey that can be pulled out from the commercially managed profitable bee colonies for further use rely on assembling surplus nectar to boost up the populace. Honey production is consequently dependent on influencing the fluctuation of foodstuffs in a hive in order to enhance the excess and perception of food accessibility. Additionally, current apprehension regarding population bees' sustainability (Neumann and Carreck, 2010) have revealed a necessity to realize that how vigorous and strong colonies function, and why they possibly and or occasionally stop working. Entire dietary requirements of bees can be managed through providing pollen and nectar collected by foragers from field (Brodschneider and Crailsheim, 2010). Nectar is completely carbohydrate in the shape of sugar (from time to time various minerals) (Kearns and Inouye, 1993; Seeley, 1993) while pollen endows the bees with lipids, protein, and vitamin and micro and macro nutrients and other minerals. Nectar is exchanged from forager to non-forager bees in the colony that accumulate in cells and, sooner or later, contemplate it to make honey. Pollen straightly accumulated in cells by foragers however it muddles up with small amount of nectar and stored by the bees. In dearth period in the areas of seasonal climate, the stored food collected by the foragers is used.

Bees have an extraordinarily distinctive blueprint regarding age polytheism conducting different duties in colony through following three weeks of their growing adult prior to changing as forager, however behavior of bees is susceptible to changes in the intensity of stored food and food incursion. Food deficiency inside the hive encourages the commencement of foraging activity in worker bees (Toth and Robinson, 2005; Schulz et al., 1998) and brood rearing is considered to be susceptible particularly. Coordination amongst flora and population fluctuation in bee colonies is multifaceted in order to find exactly the reason of bee declining. The food compilation is predisposed according to the magnitude of forager bees' population and conversely the food fluctuation through colony can manipulate/ persuade the forager strength through changing the velocity at which colony bees develop into forager bees and magnitude of the brood strength, which ultimately sooner or later will develop in to subsequent foragers' generation. Foraging has great imperativeness because survival of adults and larvae depends upon food items that the forager bees collect and bring them back in the colony.

Worker bees have capability of determining the colony requirements regarding pollen and it might be workout through the interaction in social way with the nursing bees in colony (Camazine, 1993). Worker bees those generally collect nectar; modify their foraging concentration and requirement regarding the inactive forager bees using the socially arbitrated evaluation of colony stores and the inflow. The time span that forager bees takes to be back to hive and unloaded collected food by storer bees, is a sign of food flow of colony (Seeley,1989). A famished colony make young bees to grow as a forager bees faster as compared to the bees having sufficient food (Schulz et al. 1998). Honey bees fed on *ad libitum* through feeder however released into empty honey comb, did not revealed the speedy developmental behavior which depicted that the blank honey comb is not an indication of arbitrating the intelligent foraging behavior. The current study was conducted to check the effect of food shortage in the honey bee colonies.

MATERIALS AND METHODS

Experiment-1

Four Langstroth colonies (single-cohort) were prepared and placed on iron stands with distance of 200 meters among each colony. All colonies with 1000 number of adult worker bees were taken from the same source. In this first experiment, one colony was named as a fed colony (control treatment) and other three colonies were declared as starved colonies. In control treatment, a single frame containing more than enough honey for continued existence of workers was provided and this quantity of honey was more than their requirement and endurance. While in starved colonies, single frame of honey (1.1 g per day per 100 bees).was placed that was not sufficient to feed on up to four days maximum and was actually to endure the worker bees for maximum of two days. The provided single food frames to each starved colonies were removed on day two, three or four. All bees on frames were brushed out in the hive and new pour out and blank frames were placed in all starved colonies. Same treatment of brushing the bees in to hive and replacing it with empty and pour out frame in the hive was given to fed colony as well. Foraging data collection from all colonies was started after the start of foraging activities shown by any colony for four days. Foraging data were collected twice a day at 9:00 am and 4:00 pm. foragers and non-foragers were counted manually. Mortality data in fed and starved colonies was recorded by manually counting the dead bees in the hives.

Experiment-2

In this experiment two colonies were used; one control with a single frame containing more than enough honey for workers' endurance was provided. While in starved colonies, single frame of honey (1.1 g per day per 100 bees) was placed containing the honey that was not enough to maintain it for up to four days maximum. In the evening of day 4, after the first day of foraging by any colony in the experiment, the empty food frames were removed from one of the starved colonies, and reinstate it with a frame that was full of the honey. So, this colony was nominated as the starved-to-fed ("S→F") colony. Simultaneously, the food frames were removed from one of the fed colonies and were substitute it with an empty frame and this colony was considered as the fed-to-starved (``F→S") colony. Then the other two colonies were manipulate, same as in the experiment-1, one starved colony and one fed colony.

Experiment-3

In this experiment, one as fed (control treatment), one as starved and one FBSC colonies were used. The injection was used in this experiment and filled with the 50% sugar syrup and dyed with the required blue color and was feed the whole colony. This colony was nominated as "fed bees, starved colony", FBSC. Entire experimental colony were standardized and maintained through; dying the sugar syrup with 50% in the replacement of honey using same amount to starved and fed colonies. The only food frames containing cells was reshaped so that it has only one side and the other side was having food reservoirs. Bees were observed in all colonies to check the presence of dyed food syrups in their elementary canal through causal observations. Furthermore, the presence or absence of the sugar syrup in among the honey bees was determined. The diet was applied to the colony once throughout the experiment. The foraging observations were performed almost for four days right after the start f the foraging by any colony in the experiment. Contrast of blue color of dyed food and somewhat light color of cuticle of bees was checked to ensure presence or absence of sugar syrup. Data was analyzed through calculating percent mortality of forager bees. 2×2 contingency table was also used. Furthermore the Chi-square was also used to check the significance.

RESULTS

Experiment-I

Foraging observations:

Results revealed that comparatively on average, more foragers were observed in the fed colonies than the starved colonies. There was a statistically significant difference (P = < 0.001) (See table-1) among the fed colony and starved colonies. There were more foragers in starved colony as compared to fed colonies therefore it is concluded that the food shortage in the colony can ground the sequential polytheism but not as a consequence of worker sense the shortage of food uncomplicatedly through the forager - colony dealings and interface. Temporary food shortage in colonies impinges on behavioral modifications and same has been observed in our experiment. It is revealed that one feedback of food shortage in colonies is the cannibalism, such as the adult individuals of termite's foragers and or offspring such as in honey bees (Woyke 1976, 1977).

Table 1. Comparisons about fraction of foragers and non-foragers honey bee colonies. The significance obtained from the 2×2 contingency table

Colony	Foragers	Non-foragers	Significance
Fed day 1	44	956	p-< 0.001
Fed day 2	52	948	p-< 0.001
Fed day 3	69	929	p-< 0.001
Fed day 4	78	922	p-< 0.001
Starved1 day 1	98	902	p-< 0.001
Starved1 day 2	79	921	p-< 0.001
Starved1 day 3	60	940	p-< 0.001
Starved1 day 4	57	947	p-< 0.001
sateved2 Day1	93	907	p-< 0.001
sateved2 Day2	88	912	p-< 0.001
sateved2 Day3	70	930	p-< 0.001
sateved2 Day4	65	935	p-< 0.001
satrved3 Day1	99	901	p-< 0.001
satrved3 Day2	86	914	p-< 0.001
satrved3 Day3	79	921	p-< 0.001
satrved3 Day4	66	934	p-< 0.001

Graphical representation of average foragers and non-foragers of experiment-1:

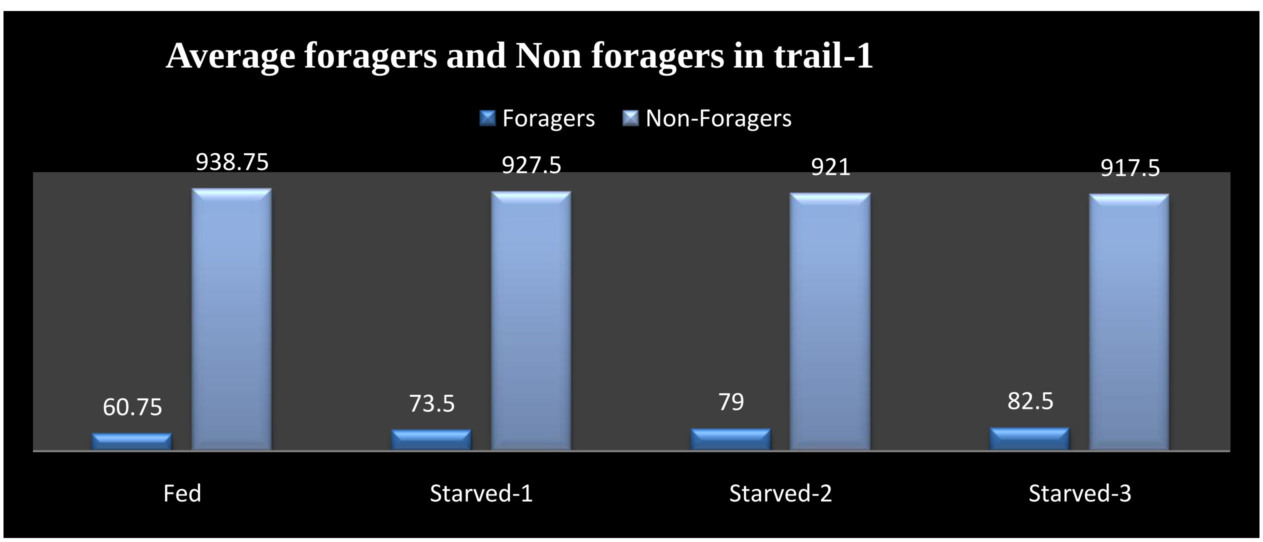

Fig.1: Average forager and non-foragers in fed and starved colonies in the trail-1.

Mortality observations:

% mortality in fed and starved colonies:

The model depicted that there was 2.01% mortality in the starved colony-1 following the starved colony-2 and starved colony-3 with the % mortality values as 7.09 and 9.95 respectively. Results revealed that as the days to the starved colony was passing; the mortality in the colonies was increasing accordingly. Overall, the mortality rate was not found so enhanced since the period was not too long and the bees become more active in order to find the flora and fauna when they comes in interaction with the colony and realize the food shortage. The following formula was used to compute the corrected % mortality. The findings are presented in below given table.

$$\text{Corrected \%} = \frac{(\text{Mortality \% in treated colony} - \text{Mortality \% in control colony}) * 100}{100 - \text{Mortality \% in control colon}}$$

Table 2. % mortality in honey bee colonies

Colonies	Mortality % in treated colony	Mortality % in control colony	Corrected % mortality
Fed	5.5	5.5	5.5
Starved-1	7.4	5.5	2.01
Starved-2	12.2	5.5	7.09
Starved-3	14.9	5.5	9.95

Calculation of the Chi-Square value:

After computing the observed and expected values, the value of qui-square was calculated in order to find the p-value using the equation;

$$X^2 = \Sigma (o - e)^2 / e$$

While;
o = observed value
e = expected value

We hypothesized that food shortage in colony causes mortality of foragers and influence the foraging activities. Results of the model revealed that the difference between observed and expected values was statistically significant as $df = 3$, $X^2 = 16.04$, $p = 0.0001$ (see table-4.3). Hence the findings support the hypothesis and revealed the mortality was also recorded more in the starved colonies comparatively with the modifications in foraging behavior. The result are in-line with Huang and Robinson (1992), who also found the behavioral modifications in the forager bees in such a way that the forager bees in the starved colonies were found as doing more foraging as compared to fed colonies.

Table 3. Calculation of Chi-Square showing probability of both observed and expected values

obs	**exp**	**o-e**	**(o-e)2**	**(o-e)2/e**
243	294.63	-51.63	-2665.656	-9.047
290	294.78	-4.78	-22.848	-0.077
316	294.78	21.22	450.288	1.527
330	294.78	35.22	1240.448	4.208
3755	3703.36	52.36	27.415	0.0074
3710	3705.213	5.213	27.175	0.0073
3684	3705.213	-21.213	-449.991	-0.1214
3670	3705.213	-35.213	-1239.955	-0.3346
			sum of column 5=	16.0434
			df=	3
			Critical value=	7.8147
			p-value=	0.0001

CHI-SQUARE CALCULATIONS

For proportion of forager and non-forager bees among colonies, chi square test was used. Results revealed that there was low mortality in the colonies and the non-foragers population could not be measureable during the trial. The chi-square model shows significant results as 16.0434 (df= 3, $p<0.001111$).

Table 4. chi-square calculations

	Colony1	**Colony 2**	**Colony 3**	**Colony4**	***Row Totals***
Foragers	243 (294.64) [9.05]	290 (294.79) [0.08]	316 (294.79) [1.53]	330 (294.79) [4.21]	1179
Non-foragers	3755 (3703.36) [0.72]	3710 (3705.21) [0.01]	3684 (3705.21) [0.12]	3670 (3705.21) [0.33]	14819
Column Totals	3998	4000	4000	4000	15998 (Grand Total)

Note:
Chi-square statistics =16.0434
p-value = 0.001111

Experiment-II

Foraging observations:

Results revealed that comparatively on average, more foragers were found in the fed colonies than in starved. Statistically significant difference among the fed and the starved colonies regarding the number of foragers and non-foragers was found (see table-4.2) which revealed that food shortage influence the colony development. It is evident that adding up of food to the combs decreases the quantity of foraging movements in forager bees (Free, 1955). Adding food to the blank and or pour out frame to bee colonies, boost up hoarding performance while the running down of pollen accumulation in colonies enhance the capacity of anthology of flora and fauna by active foragers (Fewell and Winston 1992).

Table 5. Comparisons about the fraction of foragers and non-foragers honey bee colonies. The significance obtained from the 2×2 contingency table

Colony	Forager	Non-forager	Significance
Fed1Day1	39	961	P< 0.0004
Fed1Day2	44	956	P< 0.0004
Fed1Day3	58	942	P< 0.0004
Fed1Day4	63	937	P< 0.0004
Fed2Day1	44	956	P< 0.0004
Fed2Day2	40	960	P< 0.0004
Fed2Day3	31	969	P< 0.0004
Fed2Day4	18	982	P< 0.0004
Starved1Day1	50	950	P< 0.0004
Starved1Day2	44	956	P< 0.0004
Starved1Day3	38	962	P< 0.0004
Starved1Day4	20	980	P< 0.0004
Starved2Day1	51	949	P< 0.0004
Starved2Day2	46	954	P< 0.0004
Starved2Day3	31	969	P< 0.0004
Starved2Day4	24	976	P< 0.0004

Graphical representation of average foragers and non-foragers of experiment-2:

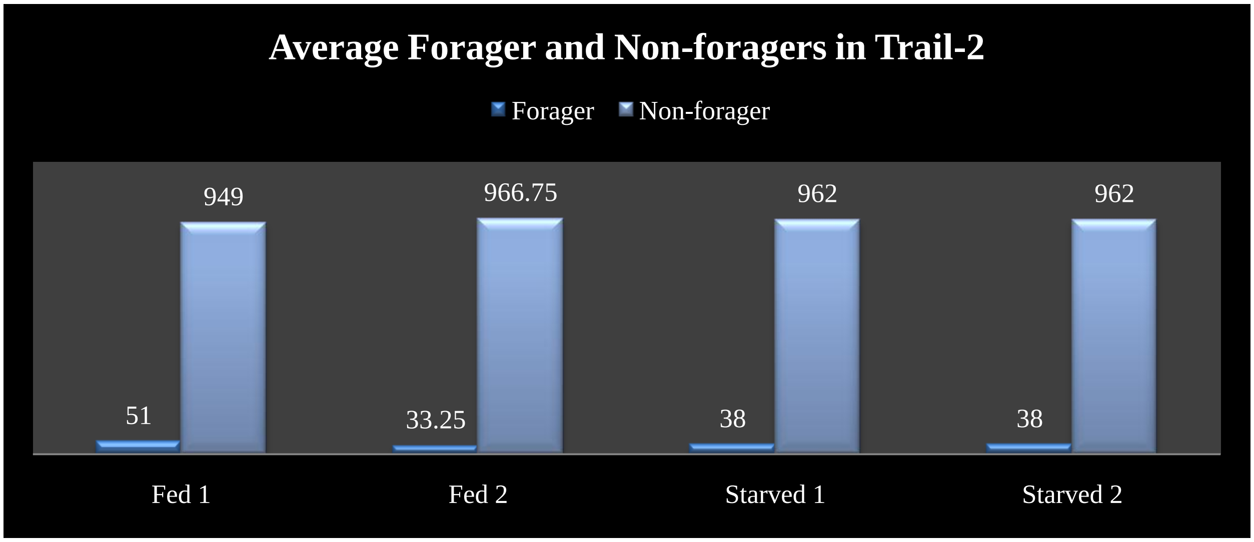

Fig.2: Average forager and non-foragers in two fed and two starved colonies in the trail-2.

Mortality observations:

% mortality in the Starved colonies:

The Schneider-Orelli's formula was used to calculate the corrected % mortality of honey bees.

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treated plot - Mortality \% in control plot}}{\text{100 - Mortality \% in control plot}} \right) * 100$$

The model revealed that there were low mortality in the starved colonies like as 3.79% in colony-1 and 4.21 in the colony-2 (see table- 4.5). As the days were prolonging, mortality was also increasing in the colonies. Less mortality was recorded in the fed colonies comparative to starved colonies.

Table 6. % mortality in the honey bee colonies used for experiment

Colonies	Mortality % in treated colony	Mortality % in control colony	Corrected % mortality
Fed-1	3.2	5.1	5.1
Fed-2	1.9	5.1	5.1
Starved-1	8.7	5.1	3.79
Starved-2	9.1	5.1	4.21

Calculation of Chi-Square value:

We hypothesized that due to shortage of food in starved colonies; there will be more mortality and less foraging activities. Results o revealed that there was a statistically significant difference among the observed and expected values as *df= 3, X2= 1448, p= 0.0004* (see table 4.6). It is concluded, and according to our hypothesis, that there was more mortality in the starved colonies as compared to fed honey bee colonies.

Table 7. Calculation of Chi-Square showing probability of observed and expected values

Obs	Exp	o-e	(o-e)2	(o-e)2/e
204	160.25	43.75	1914.0625	11.9442
133	160.25	-27.25	-742.5625	-4.6337
152	160.25	-7.75	-60.0625	-0.3748
641	160.25	480.75	231120.5625	1442.25
3796	3839.75	-43.75	-1914.0625	-0.4984
3867	3839.75	27.25	742.5625	0.1933
3848	3839.75	8.25	68.0625	0.0177
3848	3839.75	8.25	68.0625	0.0177
			sum of column 5=	1448.9131
			df=	3
			Critical value=	7.814728
			p-value=	0.0004

CHI-SQUARE CALCULATIONS

The findings revealed that there was low mortality in the colonies. The chi-square calculations were also made to check the significance as 18.1548 and the model is found significant at $p <$ 0.000409.

Table 8. Chi-square calculations

	Colony1	**colony 2**	**colony 3**	**colony4**	***Row Totals***
Foragers	204 (160.25) [11.94]	133 (160.25) [4.63]	152 (160.25) [0.42]	152 (160.25) [0.42]	641
Non-foragers	3796 (3839.75) [0.50]	3867 (3839.75) [0.19]	3848 (3839.75) [0.02]	3848 (3839.75) [0.02]	15359
Column Totals	4000	4000	4000	4000	**16000 (Grand Total)**

Experiment-III

Foraging observations:

Results revealed that on average more foragers in starved colonies (239) following the FBSC colony (206) and fed colony (160) were found. Due to food shortage in the colony, workers started foraging at higher rates and this behavior was not observed in other colonies except starved ones. Chi-square model also reported the statistically significant difference showing the P- value = 0.00001. Hence it is concluded that due to food shortage in colonies, along with mortality, majorly the colony starts immediately showing the behavioral changes, significantly the enhanced foraging activity by foragers.

Table 4.9: Comparisons about the fraction of foragers and non-foragers honey bee colonies. The significance obtained from the 2×2 contingency table

Colony	Foragers	Non-Foragers	Significance
Fed1	44	956	0.00001
Fed2	36	964	0.00001
Fed3	39	961	0.00001
Fed4	41	959	0.00001
Starved1	66	934	0.00001
Starved2	70	930	0.00001
Starved3	62	938	0.00001
Starved4	69	931	0.00001
FBSC1	50	950	0.00001
FBSC2	53	947	0.00001
FBSC3	46	954	0.00001
FBSC4	57	943	0.00001

Graphical representation of average foragers and non-foragers of trial -3:

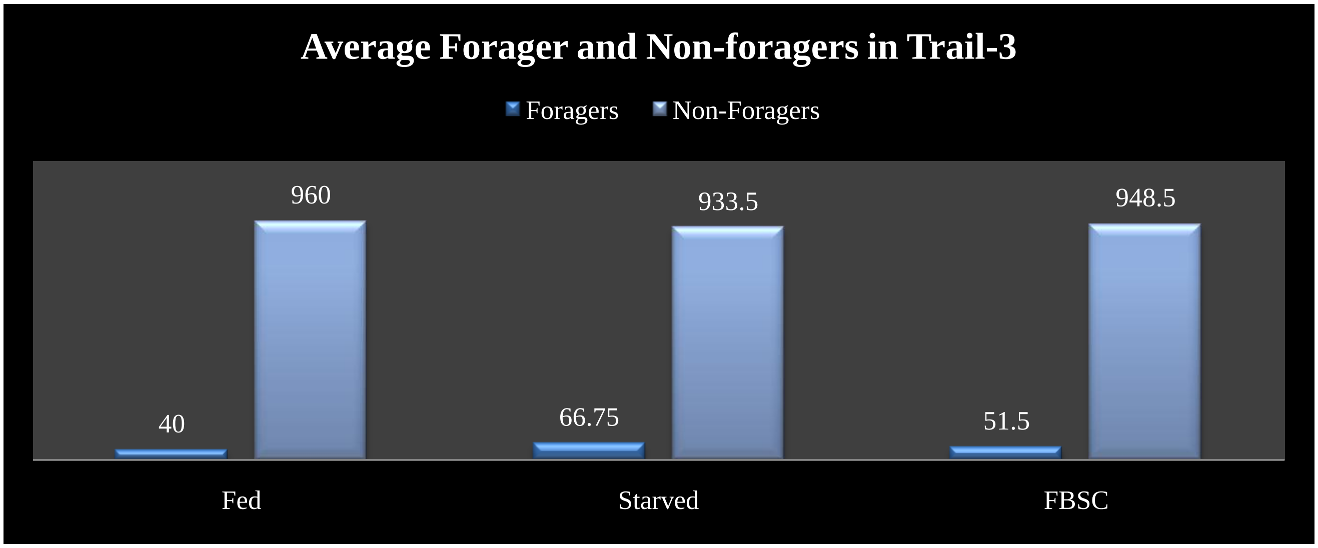

Fig.3: Average forager and non-foragers in one fed and one starved and one FBSC colonies in the trail-3

Mortality observations:

% mortality in Starved colony:

Schneider-Orelli's formula was applied in order to compute the corrected % mortality and model revealed that in fed colony, there was less mean % mortality (4.5%) as compared to starved (6.18%) and FBSC colonies (6.60 %) (table-4.8). High mortality was not observed in colonies however with the passage of time, mortality increased in treated colonies. Overall, food shortage influences the mortality and increase foraging intensity of foragers when they come in to contact with the colony and available fauna and realize food shortage. Alongside the mortality, the food scarcity also possesses the behavioral changes in the colony members.

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treated plot - Mortality \% in control plot}}{\text{100 - Mortality \% in control plot}} \right) * 100$$

Table 10. % mortality in the honey bee colonies

Colonies	Mortality % in treated colony	Mortality % in control colony	Corrected % mortality
Fed	4.5	4.5	4.5
Starved	10.4	4.5	6.18
FBSC	10.8	4.5	6.60

Chi-Square Model:

We hypothesized that due to shortage of food in starved colonies; there will be more mortality and less foraging activities. Results of qui-square model showed a statistically significant difference among the observed and expected values as the *df*=2, X^2=27.17, *p*= 0.0004 (see table-4.9). It is concluded form the findings and according to our hypothesis, that there was more mortality in the starved colonies as compared to fed honey bee colonies.

Table 11. Calculation of Chi-Square showing probability of both observed and expected values

Obs	exp	o-e	(o-e)2	(o-e)2/e
160	211	51	2601	12.3270
267	211	56	3136	14.8625
206	211	5	25	0.1184
3840	3789	51	2601	0.6864
3733	3789	-56	-3136	-0.8276
3794	3789	5	25	0.0065
			sum of column 5=	28.8288
			df=	2
			Critical value=	5.991465
			p-value=	0.00001

CHI-SQUARE CALCULATIONS

The calculations were made to check the significance and model revealed that there was low mortality in the colonies as 28.8288 and the model is found significant at $p < 0.00001$.

Table 12. Chi-square calculations for the third experiment

	Colony1	**colony 2**	**colony 3**	***Row Totals***
Foragers	160 (211.00) [12.33]	267 (211.00) [14.86]	206 (211.00) [0.12]	633
Non-foragers	3840 (3789.00) [0.69]	3733 (3789.00) [0.83]	3794 (3789.00) [0.01]	11367
Column Totals	4000	4000	4000	**12000 (Grand Total)**

Note:
Chi square statistics = 28.8288
P- value = 0.00001

Presence/ absence of the dyed food in the canals:

All the treated and control colonies with bees before the mortality and foraging observations were brought into the laboratory for the examination of the presence and or absence of dyed food in the elementary canal of the honey bees through the naked eyes under the light. It was quite easy to determine and distinguish the color combination among the honey bee canals and the sugar syrup fed to them. Our observation about the phenomenon revealed that the bees in the fed colonies and in the FBSC colonies were full of the blue color whereas the in the starved colonies, all the bees which were examined were deficient of that color.

DISCUSSION:

The behavioral effects of the food on the individual honey bees set off the corridor connected with the forager growth and development. Our findings are in-line with that model straightforwardly in presenting that the speed of behavioral improvement is not predisposed through the connection between the workers and colony; however do not endow with the additional substantiation in the support. All experiments represented were performed using Langstroth shaped, little, single-cohort colonies are pertinent findings to greater hives of honey bees. The honey bee colonies with the short population strength comparatively show more vigor and great dependency prototype of the communal behavior (von Frisch 1967; Seeley 1995).

The findings of the study recommend that in order to investigate the apparatus occupied in the improved forager bees creation because of the food shortage in honey bees, further investigations be supposed to check the consequence that colony those are deficient in having food contains no trophallaxis phenomenon. The existence and or extinction of the protein-rich diet influence the both factors; growth and improvement of the newly emerged forager bees and the movements of the prevailing foragers in the honey bee hives. The familiarity of this system is not unknown; to those colonies which can not react properly against the danger of food shortage will surely expire.

CONCLUSION:

In trail one, on average and significantly, more foragers were observed in the starved colonies than in fed ones. The temporary food shortage in the bee colonies impinges on the behavioral modifications and same has been observed in the current experiment. There was 2.01% mortality in the starved colony-1 following the starved colony-2 and starved colony-3 with the % mortality values as 7.09 and 9.95 respectively. Overall, the mortality rate was not found so enhanced since the period was not too long and the bees become more active in order to find the flora and fauna when they comes in interaction with the colony and realize the food shortage. In experiment, comparatively on average, more foragers were found in the starved colonies than the fed ones. Food provision to combs mostly decreases foraging movements similarly, adding food to the blank and or pour out frame to colonies boost up the hoarding performance. Mortality in the starved colonies also increased with the passage of time and mortality can be managed providing required food to the bee colonies. In experiment 3 comparatively and on average there was more number of foragers in starved colonies following the FBSC and fed colonies. Due to food shortage, worker bees started foraging at higher rates in starved colonies. It is observed that both the mortality and number of forager bees are influenced due to food shortage. In fed, there was less mean mortality as comparative to other colonies where it increased gradually that indicate the food shortage influence.

Conflict of interests

The author declared no conflict of interests.

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